3

DATA EVALUATION RECORD

TECHNICAL GRADE AE 0172747

Study Type: OPPTS 870.6300 [§83-6], Developmental Neurotoxicity Study in Rats

Work Assignment No. 3-1-117 Q (MRID 46695725)

Prepared for

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DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study – Rat; OPPTS 870.6300 ('83-6); OECD 426 (draft)

PC CODE: 012801 **DP BARCODE:** D325935
TXR# 0054231

TEST MATERIAL (PURITY): AE 0172747 (94% a.i.)

SYNONYMS: 2-[2-chloro-4-mesyl-3-((2,2,2-trifluoroethoxy)methyl)benzoyl]cyclohexane-1,3-dione

CITATION: Sheets, L.P., R.G. Gilmore, and H.E. Hoss (2005) A developmental neurotoxicity screening study with technical grade AE 0172747 in Wistar rats. Bayer CropScience LP, Stilwell, KS. Laboratory Study No.: 04-D72-UE, September 7, 2005. MRID 46695725. Unpublished.

SPONSOR: Bayer CropScience LP, 2 T.W. Alexander Dr, Research Triangle Park, NC

EXECUTIVE SUMMARY - In a developmental neurotoxicity study (MRID 46695725) technical grade AE 0172747 (94% a.i., Batch #s PFI 0215 and OP2250027) was administered to approximately 30 mated female Wistar rats per dose in the diet at nominal dose levels of 0, 10, 200, or 1500 ppm from gestation day (GD) 6 through lactation day (LD) 21. Doses were adjusted during lactation to achieve a more consistent dosage throughout exposure. The mean daily intake during gestation and lactation was 0, 0.8, 16.3, and 118 mg/kg/day. Dams were allowed to deliver naturally and were killed on LD 21, following weaning of their respective litters. Any females that were found to be sperm positive and/or with a vaginal plug, but did not deliver, were sacrificed on GD 24 and 8 dams and were examined for pregnancy status. On postnatal day (PND) 4, litters were standardized to 8 pups/litter; the remaining offspring and dams were sacrificed and discarded without further examinations. Subsequently, 1 pup/litter/group (at least 10 pups/sex/dose when available) were allocated to subsets for FOB, motor activity, acoustic startle response, learning and memory evaluation, and neuropathological examination. Positive control data were not submitted with this study; however, summaries of positive control data previously submitted to the Agency were obtained and reviewed.

Reserved for EPA reviewer

The maternal LOAEL is [dose] mg/kg/day, based on [endpoint]. The maternal NOAEL is [dose] mg/kg/day.

The offspring LOAEL is [dose] mg/kg/day, based on [endpoint]. The offspring NOAEL is [dose] mg/kg/day.

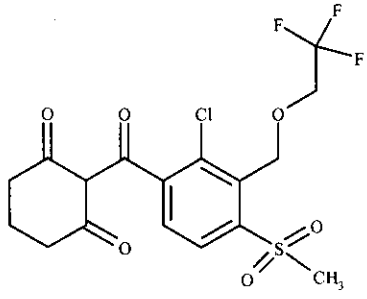
This study is classified (**acceptable/guideline**) and satisfies the guideline requirement (OPPTS 870.6300, ' 83-6); OECD 426 (draft) for a developmental neurotoxicity study in rats.

COMPLIANCE - Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** AE 0172747

Description:	Light apricot powder
Batch #s:	PFI 0215 and OP2250027
Purity:	94.0% a.i.
Stability:	The test material was shown to be stable in the diet for up to 7 days at room temperature or 35 days frozen.
CAS # of TGAI:	335104-84-2
Structure:	

2. **Vehicle** – Diet

3. **Test animals (P)**

Species:	Rat	
Strain:	Wistar Hanover Crl:WI (Glx/BRL/Han) IGS BR	
Age at study initiation:	12 weeks at cohabitation	
Mean group weights on GD 0:	217.9-221.2 g females only	
Source:	Charles River Laboratories (Raleigh, NC)	
Housing:	During gestation and lactation, individual dams and litters were kept together in plastic cages with corn cob bedding. The week following weaning, the remaining F_1 pups were kept individually in stainless steel wire-mesh cages.	
Diet:	Rodent Lab Chow #5002 (PMI Nutrition International, St. Louis MO), *ad libitum*, except during neurobehavioral testing	
Water:	Tap water, *ad libitum*, except during neurobehavioral testing	
Environmental conditions	**Temperature:**	18-26°C
	Humidity:	30-70%
	Air changes:	10/hr
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	At least 6 days	

B. PROCEDURES AND STUDY DESIGN

1. **In-life dates** - Dams received the test material starting on 5/23/04 and ending on approximately 6/28/04.

2. **Study schedule** - The maternal animals were mated and assigned to study. The test substance was administered to the dams from gestation day (GD) 6 through lactation day (LD) 21. Pups were weaned on postnatal day (PND) 21, after which time all animals received untreated diet. On PND 4, the litters were randomly standardized to 8 pups/litter

(with equal sexes where possible) to reduce the variability. All litters not selected for further observations and all P females without a litter were sacrificed, and were discarded without further examinations. F_1 pups remained on study until PND 75 (study termination).

3. **Mating procedure** - Females were paired 1:1 with males of the same strain and source for a maximum of five consecutive days. Each female was examined daily during the mating period to identify sperm cells in a vaginal smear or the presence of a copulatory plug. The day that sperm or a plug was found was designated gestation day (GD) 0, and each female was housed individually in a plastic nesting box.

4. **Animal assignment** - Time-mated females were randomly assigned to test groups as shown in Table 1. Offspring were assigned to testing subgroups at the time of litter standardization on post-natal day 4. Dams were assigned to functional observation testing as shown.

TABLE 1. Study design [a]

Experimental Parameter	Subset	Dose (ppm)			
		0	10	200	1500
Maternal Animals					
No. of dams assigned	NA	30	30	30	30
Mean daily intake (mg/kg/day)	NA	0	0.8	16.3	118 [b]
FOB (GD 13 and 20)	NA	30	30	30	30
FOB (LD 11 and 21)	NA	10	10	10	10
Offspring (F_1) [c]					
Motor activity (PND 13, 17, 21, 60±2)	A	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)
Acoustic startle habituation (PND 22, 60±2)	B	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)
Passive avoidance (PND 22 and 29)	C	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)
Water maze (PND 60±2 and 7 days later)	C	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)
FOB (PND 4, 11, 21, 35±1, 45±1, 60±2)	C	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)
Perfusion, neuropathology, and morphometric analysis (PND 21)	D	10/sex	10/sex	10/sex	10/sex
Brain weight (PND 75±5)	A, B, C	10/sex	10/sex	10/sex	10/sex
Ophthalmologic examination (PND 50-60)	A, B, C	~10/sex	~10/sex	~10/sex	~10/sex
Perfusion and neuropathology (PND 75±5)	A, B, C	same animals selected for ophthalmologic examination	same animals selected for ophthalmologic examination	same animals selected for ophthalmologic examination	same animals selected for ophthalmologic examination

a Data obtained from pages 24 and 56 of the study report.
b Excludes value from first week of measurement due to food spillage.
c Unless otherwise indicated, 1 male or female pup/litter was used (~16 [minimum of 10]/sex/dose, representing at least 20 litters).
NA Not applicable

5. **Dose selection rationale** - Dose levels were chosen based on the results of a two-generation reproduction study in Wistar rats (MRID 46695704; reviewed concurrently) and a dose range-finding study. In the two-generation reproduction study, AE0172747 was administered in the diet at nominal concentrations of 20, 200, or 1500 ppm and the LOAEL for parental and offspring toxicity was 20 ppm (equivalent to 1.4/1.6 mg/kg/day in males/females) based on effects on the eyes, including corneal opacity, acute inflammation, and neovasularization. Additionally, the following treatment-related effects were noted at 200 ppm: (i) minor decreases in parental body weight, body weight gain, and food consumption; (ii) dilated kidneys in the F1 parents of both sexes; (iii) tubular basophilia in the kidneys in the F1 male parents; (iv) decreased pup body weights and body weight gains; (v) delayed preputial separation; (vi) decreased absolute and relative spleen weights in the offspring; and (vii) increased extramedullary hematopoeisis in the offspring. It was stated that the range-finding study was conducted to provide additional information to assist in selecting an overall NOEL. In the range-finding study, AE0172747 was administered in the diet at nominal concentrations of 0, 5, 10, and 20 ppm on GD 6 through LD 21. Treatment-related effects were observed at 10 (corneal opacities in 3 dams and 1 male pup) and 20 ppm (in the dams, decreased food consumption and corneal opacities [6 dams] and in the pups, decreased body weight and body weight gain, and corneal opacity in 1 male). Based on the results of these studies, doses of 10, 200, and 1500 ppm were chosen for the current study.

6. **Dosage preparation, administration, and analysis** - Formulations were prepared weekly by mixing appropriate amounts of test substance with acetone and then with the diet. The acetone was allowed to evaporate and the test diets were stored frozen until use. Dietary formulations were provided to the dams for *ad libitum* consumption weekly throughout the exposure period (GD 6 through LD 21). F_1 animals were not directly supplied with the test diets. It was stated that in an additional dietary analysis study (MRID 46695739; reviewed concurrently), test diets were analyzed at concentrations of 3 and 3000 ppm (which bracketed the range in the current study). The same batch number of test material and type of diet (Purina Certified Rodent Chow #5002) used in the current study were used in this dietary analysis study. Stability was determined for up to 7 days at room temperature and for up to 35 days frozen and homogeneity (% relative standard deviation) at both concentrations was also determined. The results of the dietary analysis study are also provided as Appendix II in this DER. Actual concentration at each dose was tested for each batch of dietary formulations used in the current study.

Results

Homogeneity analysis (% relative standard deviation): 2.2-7.7%

Stability analysis (range as % of Day 0)

At room temperature for 7 days: 91-99%

Frozen for up to 35 days: 94-114%

Concentration analysis (range as mean % of nominal):

Dose (ppm)	% Nominal
10	88-108
200	93-108
1500	92-101

The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was acceptable.

C. OBSERVATIONS

1. In-life observations

a. Maternal animals – Cage-side checks for mortality, moribundity, and clinical signs of toxicity were conducted for at least once daily for maternal animals. Detailed clinical examinations were performed daily during exposure (GD 6 through LD 21).

Animals presumed to be pregnant (approximately 30/dose) were observed on GD 13 and 20 and a minimum of 10 dams/dose were observed on LD 11 and 21 as part of a functional observational battery (FOB). The FOB included, but was not limited to, the following observations (with severity scoring).

	FUNCTIONAL OBSERVATIONS
X	Signs of autonomic function, including: 1) Ranking or degree of lacrimation and salivation 2) Presence or absence of piloerection and exophthalmos, 3) Ranking or count of urination and defecation 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size 5) Degree of palpebral closure
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions, emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

The technicians performing the FOB were 'blind' as to the animal's treatment group. Several technicians were used during the FOB; however, it was stated that evidence of inter-observer reliability (positive control studies) is maintained at the laboratory. No further information concerning the performance of the FOB was provided.

Individual maternal body weight and food consumption data were recorded weekly throughout gestation (GD 6, 13, and 20), on the day of delivery (LD 0), and on LD 4, 7, 14, and 21.

b. Offspring

1. **Litter observations** - The day of completion of parturition was designated as PND 0. Live pups were counted, sexed and weighed individually for each litter on PND 0, 4, 11, 17, and 21. At least once daily, all surviving offspring were examined cage-side for gross signs of toxicity, mortality, or morbidity.

   On PND 4, litters were standardized (using random procedures) to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible). Pups not chosen for the F_1 groups and dams that had insufficient pups were killed and discarded without further examination.

2. **Developmental landmarks** - Beginning on PND 38, male offspring were examined daily for preputial separation. Beginning on PND 29, female offspring were examined daily for vaginal patency. The age of onset was recorded, and the pups were weighed when vaginal patency or preputial separation was first noted. In addition, all pups were tested for the presence of pupil constriction on PND 21.

3. **Post-weaning observations** - Clinical observations were recorded daily for all animals. In addition, detailed clinical observations were recorded weekly during post-weaning. Body weights were recorded weekly beginning the week of PND 28 (when pups were placed in individual housing).
4. **Neurobehavioral evaluations** - Observations and the schedule for those observations are summarized as follows from the report.

i. **Functional observational battery (FOB)** - On PND 4, 11, 21, 35 (±1 day), 45 (±1 day), and 60 (±2 days), selected pups (approximately 16/sex/dose; Subset C) were observed outside the home cage according to procedures outlined for the dams. The neonates were not evaluated in the open field on PND 4 and 11.

ii. **Motor activity testing** - Activity was evaluated in approximately 16 pups/sex/dose (Subset A) on PND 13, 17, 21, and 60 (±2 days). Motor and locomotor activity were measured by testing animals in figure eight mazes using a Columbus Instruments Universal Maze Monitoring System (Columbus, OH). Broad-spectrum background noise [74±2dB(A)] was provided, and the light intensity (100±70 Lux) over each maze was verified daily. Each test session was 60 minutes in duration, and consisted of 6 ten-minute intervals. Motor activity was measured as the number of beam interruptions that occurred during the test session. Locomotor activity was measured by eliminating consecutive counts for a given beam. Habituation was evaluated as a decrement in activity over consecutive intervals of the test session.

iii. **Acoustic startle habituation** - Acoustic startle habituation testing was performed on approximately 16 pups/sex/dose (Subset B) on PNDs 22 and 60 (±2 days). A Coulbourn Instruments Integrated Startle Response Test System (Allentown, PA) was used to conduct the test and collect the data. The test session consisted of 50 trials that began following a 5 minute adaptation period at ambient noise levels. The rats were then presented with the startle-eliciting stimulus (50 msec burst [0 msec rise/fall] of broad-spectrum 'white' noise

[~118 dB (lin)]) at 10-second intervals. The response amplitude was recorded (maximum value on the curve) and the baseline (animal's body weight) was subtracted.

iv. **Learning and memory testing** - Learning and memory testing was performed on approximately 16 pups/sex/dose (Subset C). Passive avoidance testing was performed on PNDs 22 and 29; water maze testing was performed on PND 60 (±2 days) and again seven days later. For both tests, only animals that demonstrated acquisition on the first day were tested for retention seven days later.

Passive avoidance test - The test was conducted using a Coulbourn Instruments Shuttle Cage System (Allentown, PA). Each shuttle cage consisted of two equal sized compartments separated by a wall that supported a (guillotine-type) door. The walls of one compartment were covered with black film (dark-side), and the other compartment was illuminated with a high intensity lamp. The floor of the dark-side consisted of a grid of stainless-steel bars. Movement of the animal across the doorway was detected with a photocell system. A Coulbourn solid state scanning shock generator was used to deliver a brief (0.5 sec) pulse of mild (0.5 mA) shock to the grid floor when the animal crossed into the dark compartment. After adaptation, individual animals were placed into the lighted compartment of a conditioning apparatus facing toward the light. After approximately 60 seconds, the trial began with the light being illuminated to signal the beginning of the trial and the door separating the two compartments opening, so that each rat was provided access to the dark-side of the cage. When the rat crossed into the dark compartment, the door automatically closed, the shock was delivered, and the light was switched off, signaling the end of the trial. At that time the animal was returned to the holding cage to await the next trial. If the rat failed to cross within 180 seconds, it was returned to the holding cage and the latency assigned an arbitrary score of 180. The procedure was repeated until either the rat remained in the lighted compartment for 180 seconds on two consecutive trials or until 15 trials had elapsed, whichever occurred first. Rats that failed to meet the criterion during the learning phase were assigned a value of 15 for the trials-to-criterion variable. The test was repeated one week later. For the second trial, rats were placed in the illuminated side of the apparatus, given a 60 second acclimation period, and the latency to enter the dark side was recorded. Animals that either failed to reach criterion within 15 trials, or failed to cross during the first two trials during acquisition, were excluded from the retention phase of the experiment.

Water maze - A Plexiglas M-maze containing 7.5 inches of water (22±1EC) was used. On each test trial, the rat was placed into the starting position at the base of the M-maze stem, located between the two lateral arms. On the first trial (learning trial), the rat was required to enter both arms of the maze before being provided access to the exit ramp to escape the water and then removed from the maze. The initial arm chosen on this learning trial was designated the incorrect goal during the subsequent 15 trials (maximum). Rats that failed to make a correct goal choice within 60 seconds in any given trial were guided to the correct goal with the exit ramp and then removed from the water. Between trials, the animal was returned to a transport cage to wait for the next trial. The inter-trial interval was approximately 15 (±5) seconds. Each rat was required to reach a criterion of five consecutive errorless trials to terminate the test session. The maximum number of trials in any test session was fifteen. Latency to choose the correct goal or the maximum 60-second interval

was recorded for each trial, as was the number of errors during each trial. Animals that satisfied the above criteria within the 15 trial limit were tested for retention seven days following acquisition. Animals that failed to reach criterion during acquisition were excluded from the retention phase of the experiment. The correct goal and criterion were the same in both sessions.

5. **Ophthalmology** - Animals that were selected for perfusion (minimum of 10/sex/dose) were subjected to ophthalmoscopic examinations at approximately 50-60 days of age. The eyes of each animal were examined with a slit lamp microscope and an indirect ophthalmoscope equipped with a condensing lens.

6. **Cholinesterase determination** – Cholinesterase activity was not determined.

7. **Postmortem observations**

a. **Maternal animals** - Maternal animals were sacrificed by carbon dioxide asphyxiation on either GD 24 (rats that did not deliver) or LD 21 (following weaning). Gross necropsies were performed on 8 dams (4 low dose, 1 mid dose, 3 high dose) that did not deliver and pregnancy status was determined. All other dams were sacrificed without necropsy.

b. **Offspring** - The offspring selected for perfusion on PND 21 (subset D) and at study termination (subsets A-C), as well as those selected for fresh brain weight determinations (approximately 10/sex/group from subsets A-C) were examined grossly.

The animals selected for perfusion on PND 21 (Subset D) and at termination (Subsets A-C) were anaesthetized with pentobarbital (50 mg/kg i.p.), and then perfused with a buffered sodium nitrite flush followed by *in situ* fixation with 1.0% (w/v) glutaraldehyde and 4% (w/v) formaldehyde in phosphate buffer. Only the brain (with olfactory bulbs) was collected from the perfused animals on PND 21. Upon study termination, the brain and spinal cord, eyes (with optic nerves), selected peripheral nerves (sciatic, tibial, and sural), the gasserian ganglion, gastrocnemius muscle, and both forelimbs were collected. All tissues were post-fixed in 10% buffered formalin. The brain from each animal was weighed, sectioned (8 coronal sections), and examined microscopically. The brain, spinal cord, cauda equina, eyes, optic nerves, and gastrocnemius muscle were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. All remaining tissues were embedded in resin, sectioned, and stained with modified Lee's stain. Additionally, the brain sections selected for morphometric measurements were stained with Luxol fast blue/cresyl violet. Additionally, the following (CHECKED X) tissues, to be examined microscopically, were collected from perfused animals at study termination:

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN	X	**SCIATIC NERVE**
X	Olfactory bulb region		Proximal
X	Olfactory bulb section		Distal
X	Forebrain (optic nerve) section		
X	Forebrain (optic chiasma) section		**OTHER**
X	Midbrain section	X	Sural nerve
X	Mesencephalon	X	Tibial nerve
X	Cerebellum/Pons		Peroneal nerve
X	Medulla oblongata	X	Cervical dorsal root ganglion
	SPINAL CORD	X	Cervical dorsal root fibers
X	Cervical swelling	X	Cervical ventral root fibers
X	Thoracic swelling		Thoracic dorsal root ganglion
X	Lumbar swelling		Thoracic dorsal root fibers
	OTHER		Thoracic ventral root fibers
X	Gasserian ganglion	X	Lumbar dorsal root ganglion
	Pituitary gland	X	Lumbar dorsal root fibers
X	Eyes (with optic nerve)	X	Lumbar ventral root fibers
X	Skeletal muscle (gastrocnemius)		
X	Cauda equina		

Only tissues from the control and 1500 ppm groups were subjected to microscopic examination and morphometric analysis. Prior to sectioning, the anterior to posterior length of the cerebrum and cerebellum were measured. The following brain sections were measured: 1) frontal cortex thickness; 2) parietal cortex thickness; 3) caudate putamen horizontal width; 4) hippocampal gyrus thickness; and 5) cerebellum height.

D. DATA ANALYSIS

1. **Statistical analyses** – In general, continuous data were initially assessed for equality of variance using Bartlett's test. Group means with equal variances were analyzed further using ANOVA, followed by Dunnett's test as necessary. Group means with unequal variances were analyzed using non-parametric procedures (Kruskal-Wallis ANOVA followed by the Mann-Whitney U test). The level of significance was set at p#0.05, with the exception of Bartlett's test which was set at p#0.001. The following data sets were analyzed by specific statistical procedures:

Parameter	Statistical Procedure
FOB continuous data, motor and locomotor total session activity, acoustic startle response amplitude data (peak amplitude)	ANOVA followed by Dunnett's, as necessary
FOB categorical data	General Linear Modeling and Categorical Modeling Dunnett's test and Analysis of Contrasts
Interval motor and locomotor activity data	Repeated measures ANOVA (test interval and test occasion) followed by ANOVA and Dunnett's, as necessary
Acoustic startle response amplitude, block data	Repeated measures ANOVA (test block) followed by Dunnett's, as necessary
Passive avoidance (latency data)	Wilcoxon Test for time to failure
Passive avoidance (number of trials-to-criterion) Water maze (number of trials-to-criterion and number of errors)	Kruskal-Wallis and Wilcoxon tests for the acquisition phase and Fisher's Exact Test for retention
Water maze (latency data)	Univariate ANOVA followed by Dunnett's, as necessary
Brain weight, gross brain measurements	ANOVA (multiple-group comparisons) or Kruskal-Wallis
Microscopic brain measurements	ANOVA and/or 2-tailed T-test (two-group comparisons)
Micropathology	Chi-Square One-tailed Fisher's Exact test

The reviewers consider the statistical methods to be appropriate.

2. Indices

a. Reproductive indices - The following reproductive indices were calculated from breeding and parturition records of animals in the study:

Mating index = # inseminated females/# females co-housed with males x 100

Fertility index = # pregnant females/# inseminated females x 100

b. Offspring viability indices - The following viability (survival) indices were calculated from lactation records of litters in the study:

Live birth index = # live pups born per litter/total # pups per litter x 100

Viability index = # live pups on PND 4 pre-culling per litter/# live pups born per litter x 100

Lactation index = # live pups on PND 21 per litter/# live pups on PND 4 post-culling per litter x 100

3. **Positive and historical control data** - It was stated that previous studies (MRIDs 42770301 and 45464602) with untreated animals and rats treated with substances that increase (triadimefon) and decrease (chlorpromazine) motor activity have established the sensitivity, reliability, and validity of the test procedures. Additional studies (MRID 45441302) have been performed to establish test norms for the appropriate ages under these conditions and the effects of perinatal exposure to a reference chemical (methimazole) on activity in animals tested at these ages. Further studies (MRIDs 45464601 and 45441303) were performed to validate the procedures and observers of the performing lab to conduct the FOB, auditory startle, passive avoidance, and water maze tests. The data from these referenced studies were not provided with the current study; however, summaries of the data from MRIDs 45464601, 45464602, 45441302, and 45441303, previously submitted to the Agency, were obtained and reviewed, and are included as Appendix I to this DER. It should be noted that the positive control data have been determined, by the Agency, to be marginal to inadequate.

II. RESULTS

A. PARENTAL ANIMALS

1. **Mortality and clinical and functional observations** - No treatment-related mortalities were observed during the study. One 1500 ppm dam (# 3122) was found dead on GD 15. This death was not attributed to treatment because this was the only animal that died or had signs of moribundity (paleness and cold-to-the-touch). All other dams survived to scheduled sacrifice.

   In the 1500 ppm dams, clinical signs of toxicity were limited to discolored stool (1-4 dams between GD 10-20), hunched posture (1 dam between GD 18-20), and unthrifty appearance (1-2 dams between GD 13-15) during gestation (Table 2). During lactation, clinical signs of toxicity were limited to corneal opacity in the 200 (3-19 dams between LD 6-21) and 1500 ppm dams (5-19 dams between LD 6-21).

TABLE 2. Selected clinical signs (maximum # affected) in dams exposed to AE 0172747 in the diet from GD 6 through LD 21. [a]

Observation	Dose (ppm)			
	0	10	200	1500
Gestation period				
Discolored stool	0	0	0	4
Hunched posture	0	0	0	1
Unthrifty appearance	0	0	0	2
Lactation period				
Corneal opacity	0	0	19	19

a Data were extracted from Tables 2 and 5 on pages 58, 59, and 65 of the study report; n=20-23.

Treatment-related FOB observations (# affected/10 vs. 0/10 controls) were limited to unthrifty appearance and impaired gait in one 1500 ppm dam (animal #3107) on GD 13 and corneal opacity at 200 (6-9) and 1500 ppm (9-10) on LD 11 and 21 (Table 3). It was stated that the severity of the corneal opacity precluded determination of pupil size and response assessments in 2-3 dams at 200 and 1500 ppm. No treatment-related observations were noted on GD 20.

TABLE 3. Selected FOB observations (# affected) in dams exposed to AE 0172747 in the diet from GD 6 through LD 21. [a]

Observation	Dose (ppm)			
	0	10	200	1500
GD 13				
Unthrifty appearance	0	0	0	1
Impaired gait	0	0	0	1
LD 11				
Corneal opacity	0	0	6	9
LD 21				
Corneal opacity	0	0	9	10

a Data were extracted from Table 16 on pages 98-121 of the study report; n=30 on GD 13 and n=10 on LD 11 and 21.

2. **Body weight and food consumption** - Selected group mean body weights, body weight gains, and food consumption for pregnant and nursing dams are summarized in Table 4.

At 200 ppm and above, body weight in the dams was decreased ($p \leq 0.05$) by 5-7% on GD 13 and 20 and by 3-5% during LD 0 through 14. However, no statistically significant differences in body weight were noted on LD 21. Overall (GD 0-20) body weight gain during gestation was decreased ($p \leq 0.01$) by 13-17% in the 200 ppm and above dams. Overall body weight gain during lactation was similar to controls at all doses.

During gestation, no treatment-related differences in food consumption were observed. The decrease ($p \leq 0.01$) noted in the 10 ppm group during GD 6-13 was not dose-dependent, and the 111% increase ($p \leq 0.01$) noted in the 1500 ppm group during GD 6-13 was attributed to spillage. During lactation, food consumption was decreased ($p \leq 0.01$) by 8-12% in the 200 ppm and above dams during LD 7-14 and remained decreased by 12% in the 1500 ppm dams during LD 14-21. These decreases in food consumption corresponded with the decreases observed in body weight during the lactation period.

TABLE 4. Selected mean (±SD) body weight, body weight gain, and food consumption in dams exposed to AE 0172747 in the diet from GD 6 through LD 21. [a]

Observations	Dose (ppm)			
	0	10	200	1500
Gestation (n=25-30)				
Body weight (g)				
GD 0	221.2±1.56	219.9±1.42	217.9±1.65	220.8±1.45
GD 13	268.6±2.36	263.0±2.05	256.4±1.83**(↓5)	250.7±2.99**(↓7)
GD 20	335.2±3.48	325.7±3.95	317.4±4.28**(↓5)	315.7±3.42**(↓6)
Body weight gain (g)				
GD 0-20	114.1±2.50	105.7±3.38	99.5±3.73**(↓13)	94.9±2.57**(↓17)
Food consumption (g/animal/day)				
GD 6-13	21.3±0.45	19.4±0.30**(↓9)	21.9±1.30	45.0±5.10**(↑111)
GD 13-20	23.4±0.41	22.0±0.51	22.9±0.66	24.9±0.75
Lactation (n=19-29)				
Body weight (g)				
LD 0	259.0±2.25	252.3±2.74	246.9±2.53**(↓5)	246.6±2.72**(↓5)
LD 14	298.2±2.87	296.0±2.23	288.7±1.86*(↓3)	281.9±3.47**(↓5)
LD 21	286.9±3.47	287.0±2.04	280.6±2.47	277.0±3.12
Body weight gain (g)				
LD 0-21[b]	27.9	34.7	33.7	30.4
Food consumption (g/animal/day)				
LD 0-7	38.6±1.40	40.2±2.10	35.6±1.13	35.0±1.24
LD 7-14	55.4±0.93	52.9±0.84	51.2±0.80**(↓8)	48.6±1.12**(↓12)
LD 14-21	64.7±1.26	65.7±0.89	64.9±1.34	57.0±1.00**(↓12)

a Data were extracted from Tables 3, 4, 6, and 7 on pages 61, 63, 68, and 70 of the study report. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).
b Calculated by reviewers from data within this table
* Statistically significantly different from controls at $p \leq 0.05$
** Statistically significantly different from controls at $p \leq 0.01$

3. **Test substance intake** - Mean daily mg test substance/kg body weight during the gestation and lactation periods are presented in Table 5. Intake was based on maternal food consumption, body weight, and nominal dose.

TABLE 5. Test substance intake (mg/kg/day) in dams exposed to AE 0172747 in the diet from GD 6 through LD 21. [a]

Interval	Dose (ppm)			
	0	10	200	1500
Gestation (n=24-30)				
GD 6-13	0.0±0.00	0.8±0.01	17.5±1.10	289.2±34.67
GD 13-20	0.0±0.00	0.9±0.02	16.6±0.49	150.6±5.27
Lactation (n=20-23)				
LD 0-7	0.0±0.00	0.9±0.05	16.2±0.54	108.2±3.49
LD 7-14	0.0±0.00	0.7±0.01	15.7±0.24	109.2±1.70
LD 14-21	0.0±0.00	0.7±0.01	15.7±0.34	102.5±1.31

a Data were extracted from Table 8 on pages 72-73 of the study report.

4. **Reproductive performance** – Reproductive parameters were not affected by treatment (Table 6). Although the median number of gestation days was increased ($p \leq 0.05$) at all doses (22.0 treated vs. 21.0 controls), this finding was considered incidental since there was no dose-dependent effect, there was no effect on this parameter in the two-generation reproduction study (MRID 46695704; reviewed concurrently) at 1500 ppm, and it was stated that 22.0 days is more typical for median gestation length for control Wistar rats in development neurotoxicity studies conducted at the performing laboratory (no historical data were provided).

TABLE 6. Reproductive performance [a]

Observation	Dose (ppm)			
	0	10	200	1500
Number mated	30	30	30	30
Mating index (%)[b]	100.0	100.0	100.0	100.0
Fertility index (%)[b]	100.0	86.7	96.7	90.0
Gestation length (median # of days)	21.0	22.0*	22.0*	22.0*

a Data were extracted from Table 1 on page 56 of the study report.
b **Mating index** = # inseminated females/# females co-housed with males x 100 and **fertility index** = # pregnant females/# inseminated females x 100
* Statistically significantly different from controls at $p \leq 0.05$

5. **Maternal postmortem results** – Since there was a somewhat high number of treated dams that did not deliver by GD 24, a gross necropsy was performed (animal #s 1101, 1116, 1124, 1126, 2115, 3123, 3127, and 3128) to determine their status. Six of these dams were found not to be pregnant while the remaining two dams had one dead pup (1116) or one implantation site (3123). These results did not indicate a treatment-related effect.

B. OFFSPRING

1. **Viability and clinical signs** - Litter size and viability results from pups during lactation are summarized in Table 7. No compound related effects on any litter parameter were noted at any dose. Live birth, viability, and lactation indices were similar to controls at all doses.

TABLE 7. Litter size and viability [a]				
Observation	Dose (ppm)			
	0	10	200	1500
No. of litters	23	22	23	20
Total number of pups born	285	258	259	233
Number born dead	2	1	0	3
Sex Ratio Day 0 (% %)	NR	NR	NR	NR
# Deaths Days 0-4 (%)	0	1	3	6
# Deaths Days 4-21 (%)	0	0	1	4
Mean litter size:				
PND 0	12	12	11	12
PND 4 [b]	12	12	11	11
PND 4 [c]	8	8	8	8
PND 21	8	8	8	8
Live birth index (%) [d]	99.4	99.7	100.0	98.7
Viability index (%) [d]	98.7	98.3	97.4	98.3
Lactation index (%) [d]	100.0	100.0	99.5	98.8

a Data were extracted from page 43 and Table 9 on pages 75-76 of the study report.
b Pre-culling
c Post-culling
d **Live birth index** = # live pups born per litter/total # pups per litter x 100; **viability index** = # live pups on PND 4 pre-culling per litter/# live pups born per litter x 100; and **lactation index** = # live pups on PND 21 per litter/# live pups on PND 4 post-culling per litter x 100
NR Not reported

No clinical signs of toxicity were observed during pre-weaning (PND 0-21) at any dose in either sex. During post-weaning, increased incidence of corneal opacity was noted in F_1 pups at 200 (2 males and 1 female) and 1500 ppm (5 males, Table 8). Opacity was observed between PND 29-71 in the males and 37-44 in the female.

TABLE 8. Incidence (# affected/8) of corneal opacity in F_1 pups during post weaning. [a]							
Dose (ppm)							
0	10	200	1500	0	10	200	1500
Males (n=58-69)				Females (n=59-69)			
0	0	2	5	0	0	1	0

a Data were extracted from Table 11 on pages 81-82 of the study report.

2. **Body weight** – Offspring pre-weaning body weights were decreased ($p \leq 0.01$) at 200 (↓7-9% both sexes) and 1500 ppm (↓9-14% both sexes) from PND 11-21 (Table 9a). Body weight gains (Table 9b) were decreased ($p \leq 0.05$) throughout most pre-weaning intervals at 200 (↓8-13%, males and ↓9-14%, females) and 1500 ppm (↓10-27%, males and ↓11-32%, females). Overall combined pup body weight gains (calculated by reviewers) were decreased by 10-16% at 200 ppm and above.

TABLE 9a. Mean (±SE) pre-weaning F_1 pup body weights (g) [a]

Postnatal Day	Dose (ppm)			
	0	10	200	1500
		Males		
0	5.8±0.09	6.0±0.11	6.1±0.07	6.1±0.08
4[b]	9.4±0.20	9.5±0.23	9.3±0.13	9.0±0.22
4[c]	9.4±0.20	9.6±0.23	9.3±0.14	9.0±0.23
11	25.2±0.43	24.5±0.47	23.0±0.50**(↓9)	22.8±0.54**(↓10)
17	39.3±0.64	38.5±0.59	36.5±0.59**(↓7)	35.5±0.78**(↓10)
21	49.5±0.95	48.8±0.72	45.3±0.73**(↓8)	42.9±0.83**(↓13)
		Females		
0	5.5±0.09	5.8±0.10	5.7±0.07	5.7±0.09
4[b]	9.1±0.20	9.2±0.24	8.9±0.12	8.6±0.22
4[c]	9.1±0.20	9.2±0.24	8.9±0.12	8.7±0.23
11	24.3±0.41	24.0±0.50	22.4±0.41**(↓8)	21.8±0.55**(↓10)
17	38.1±0.59	37.6±0.62	35.4±0.58**(↓7)	34.5±0.73**(↓9)
21	47.9±0.88	47.6±0.73	43.9±0.57**(↓8)	41.2±0.76**(↓14)

a Data were extracted from Table 12 on pages 84-86 of the study report; n=20-23 litters. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).
b Pre-culling
c Post-culling
** Statistically significantly different from controls at $p \leq 0.01$

TABLE 9b. Selected mean (±SD) pre-weaning F_1 pup body weight gains (g) [a]

Interval (PND)	Dose (ppm)			
	0	10	200	1500
		Males		
0-4	3.6±0.14	3.5±0.16	3.2±0.13	3.0±0.17*(↓17)
11-17	14.2±0.36	13.9±0.28	13.6±0.27	12.8±0.32*(↓10)
11-21	24.3±0.59	24.3±0.36	22.3±0.41*(↓8)	20.2±0.42**(↓17)
17-21	10.1±0.46	10.3±0.34	8.8±0.28*(↓13)	7.4±0.21**(↓27)
		Females		
0-4	3.6±0.13	3.5±0.19	3.2±0.10*(↓11)	2.9±0.16**(↓19)
4-17	29.0±0.50	28.4±0.50	26.5±0.60**(↓9)	25.8±0.57**(↓11)
17-21	9.8±0.37	10.0±0.24	8.4±0.24**(↓14)	6.7±0.27**(↓32)
		Combined		
Overall (0-21) gain [b]	43.0	42.3	38.7 (↓10)	36.2 (↓16)

a Data were extracted from Table 13 on pages 88-91 of the study report; n=20-23 litters. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).
b Calculated by reviewers using combined data from Table 12 on pages 84-86 of the study report
* Statistically significantly different from controls at $p \leq 0.05$
** Statistically significantly different from controls at $p \leq 0.01$

Offspring post-weaning body weights remained decreased ($p \leq 0.05$) in the males throughout the study at 200 (↓9-14%) and 1500 ppm (↓9-16%) and in the females for all but the last 5 weeks of the study at 200 (↓5-12%) and 1500 ppm (↓6-16%, Table 10). It was stated that the decreases ($p \leq 0.05$) in body weight noted in the 10 ppm males (↓5-6%) were considered unrelated to treatment because they were minor, only observed in one sex, occurred many weeks after treatment was discontinued, and the weights were within the range of historical controls (no data provided).

TABLE 10. Selected mean (±SD) post-weaning F_1 pup body weights (g) [a]				
Post-weaning Week	Dose (ppm)			
	0	10	200	1500
Males				
0	82.0±6.8	78.1±8.1	70.8±5.2*(↓14)	68.5±4.8*(↓16)
5	215.8±13.7	205.8±14.8*(↓5)	192.4±11.2*(↓11)	193.4±11.5*(↓10)
7	294.4±17.0	277.6±18.7*(↓6)	266.6±16.0*(↓9)	265.8±17.8*(↓10)
10	324.2±19.9	303.8±21.5*(↓6)	293.2±17.8*(↓10)	295.3±19.6*(↓9)
Females				
0	78.7±5.3	75.2±7.7	69.1±4.1*(↓12)	65.9±5.7*(↓16)
5	156.1±6.7	151.4±9.7	148.2±4.7*(↓5)	146.6±12.4*(↓6)
10	195.7±8.8	193.8±11.5	190.9±8.8	192.1±17.0

a Data were extracted from Table 15 on pages 95-96 of the study report; n=20-23 litters. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).

* Statistically significantly different from controls at $p \leq 0.05$

3. Developmental landmarks

a. Sexual maturation – No treatment-related effects on sexual maturation were observed in either sex (Table 11). Although the day of preputial separation was delayed ($p \leq 0.01$) in the 1500 ppm males (45.9 treated vs. 43.8 controls), this delay was considered to be related to the significantly decreased body weights in these animals rather than a direct effect of the test material. This finding is consistent with the results from the two-generation reproduction study (MRID 46695704; reviewed concurrently) at similar doses. All pups displayed pupil constriction on PND 21.

TABLE 11. Sexual maturation (mean ±SE day of onset) in F_1 pups [a]				
Parameter	Dose (ppm)			
	0	10	200	1500
Preputial separation	43.8±0.40	43.0±0.37	44.9±0.31	45.9±0.38**
Vaginal patency	33.5±0.25	34.0±0.58	35.0±0.48	34.8±0.71

a Data were extracted from Table 14 on page 93 of the study report; n=20-23 litters.

** Statistically significantly different from controls at $p \leq 0.01$

b. Physical landmarks – Evaluation of physical landmarks (eye opening, pinna unfolding, incisor erupting) was not performed.

4. Behavioral assessments

a. Functional observational battery – Treatment-related FOB effects were limited to corneal opacity at 200 (1 pup each sex) and 1500 ppm (2 males, Table 12). The opacity was noted beginning on PND 35 in both sexes. All other FOB findings were considered incidental and unrelated to treatment.

TABLE 12. Incidence (# affected) of corneal opacity in F_1 pups [a]								
	Dose (ppm)							
Post-natal Day	0	10	200	1500	0	10	200	1500
	Males				Females			
4	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0
35	0	0	1	1	0	0	1	0
45	0	0	1	2	0	0	1	0
60	0	0	1	1	0	0	0	0

a Data were extracted from Table 17 on pages 123-182 of the study report; n=15-16 pups from at least 20 litters/dose.

b. Motor activity – No treatment-related differences in total session motor or locomotor activity were observed at any dose in either sex (Tables 13a and 13b). Statistically significant differences ($p \leq 0.05$) in interval activity were limited to a decrease in motor activity in the 10 ppm females during Interval 5 on PND 17 and an increase in locomotor activity in the 200 ppm females during Interval 6 on PND 21. These findings were not considered to be treatment-related as they were not dose-related and occurred at only one interval. Levels of activity progressively increased with age compared to levels on PND 13. Habituation was evident in both sexes at all ages except for locomotor activity on PND 13, when locomotor activity levels were low during the first intervals.

TABLE 13a. Mean (±SD) total session motor activity (counts) in F1 pups [a]				
Interval (PND)	Dose (ppm)			
	0	10	200	1500
Males				
13	47±35	75±44	74±59	87±48
17	183±130	192±127	158±96	168±125
21	273±148	313±85	283±115	295±98
60	502±117	482±130	595±134	528±163
Females				
13	58±52	53±39	72±45	57±43
17	185±126	169±96	214±129	202±106
21	257±73	258±92	274±99	303±90
60	804±214	722±142	666±198	750±218

a Data were extracted from Table 18 on pages 184-185 of the study report; n=15-16 pups from at least 20 litters/dose.

TABLE 13b. Mean (±SD) total session locomotor activity (counts) in F1 pups [a]				
Interval (PND)	Dose (ppm)			
	0	10	200	1500
Males				
13	4±3	6±5	3±2	4±4
17	38±34	44±29	32±23	29±27
21	77±38	98±33	96±35	83±31
60	349±96	319±116	395±101	358±124
Females				
13	7±9	5±5	5±4	3±3
17	48±39	45±26	53±39	38±26
21	80±21	83±27	84±40	97±33
60	519±191	478±93	425±151	500±151

a Data were extracted from Table 19 on pages 187-188 of the study report; n=15-16 pups from at least 20 litters/dose.

c. **Auditory startle reflex habituation** – No treatment-related effect on total session peak amplitude, latency, or habituation were observed in either sex at PND 22 (Table 14). On PND 60, peak amplitudes were decreased ($p \leq 0.05$) in the males at 200 (↓50%) and 1500 ppm (↓45%). Although this finding did not display a clear dose-response, it was considered to be compound-related based on the magnitude of the decreases, which were below the range of the historical controls. No treatment-related effect on amplitude, latency, or habituation were observed in the females at PND 60. Interval values for all parameters were similar to controls in both sexes on PND 22 and in the females on PND 60 (Tables 15a and 15b). In the males on PND 60, the interval peak amplitude values were decreased ($p \leq 0.05$) by 45-54% during all 5 blocks at 200 ppm and by 43-50% during the first 3 blocks at 1500 ppm.

TABLE 14. Mean (±SD) overall (Blocks 1-5) acoustic startle peak amplitude (g) and latency to peak (msec) in F_1 rats [a]					
Dose (ppm)	Parameter	Males		Females	
		PND 22	PND 60	PND 22	PND 60
0	Peak Amp.	31±21	270±112	27±10	122±69
	Latency	39±5	40±2	39±3	42±4
10	Peak Amp.	34±11	202±106	28±15	120±67
	Latency	37±3	39±3	40±5	39±3
200	Peak Amp.	21±7	136±87*(↓50)	21±7	103±72
	Latency	38±4	38±2	39±4	39±6
1500	Peak Amp.	22±8	149±109*(↓45)	24±9	100±118
	Latency	40±4	39±3	41±5	39±3

a Data were extracted from Table 22 on pages 208-209 of the study report; n=15-16. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).

* Statistically significantly different from controls at $p \leq 0.05$

TABLE 15a. Mean (±SD) interval acoustic startle peak amplitude (g) and latency to peak (msec) in male F_1 rats [a]

Dose (ppm)	Parameter	Block 1	Block 2	Block 3	Block 4	Block 5
PND 22						
0	Peak Amp.	30±20	36±24	32±23	30±21	28±18
	Latency	38±5	37±7	39±7	38±7	40±9
10	Peak Amp.	35±12	37±14	35±14	33±12	30±11
	Latency	37±4	36±4	37±5	36±3	37±5
200	Peak Amp.	24±12	23±8	23±6	19±8	18±5
	Latency	39±5	39±6	38±4	37±6	38±7
1500	Peak Amp.	26±7	22±10	22±11	20±9	21±10
	Latency	43±7	40±6	39±5	38±5	40±5
PND 60						
0	Peak Amp.	336±155	367±179	268±154	211±82	170±80
	Latency	42±3	41±3	39±2	39±3	39±3
10	Peak Amp.	248±119	228±127*(↓38)	189±123	177±113	170±90
	Latency	40±3	41±3	40±4	38±3	38±5
200	Peak Amp.	181±95*(↓46)	171±121*(↓53)	122±93*(↓54)	114±98*(↓46)	94±66*(↓45)
	Latency	41±3	39±3	38±3	38±3	37±3
1500	Peak Amp.	190±146*(↓43)	185±157*(↓50)	135±82*(↓50)	128±115	108±86
	Latency	41±4	39±4	37±3	37±3	39±4

a Data were extracted from Table 23 on pages 211-212 of the study report; n=15-16. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).

* Statistically significantly different from controls at $p \leq 0.05$

TABLE 15b. Mean (±SD) interval acoustic startle peak amplitude (g) and latency to peak (msec) in female F_1 rats [a]

Dose (ppm)	Parameter	Block 1	Block 2	Block 3	Block 4	Block 5
PND 22						
0	Peak Amp.	31±19	29±10	26±10	26±11	22±9
	Latency	40±5	39±8	40±5	38±5	40±5
10	Peak Amp.	28±18	31±16	31±17	24±15	25±13
	Latency	39±5	38±5	41±8	40±9	40±8
200	Peak Amp.	24±12	24±9	22±6	20±7	17±7
	Latency	40±8	40±8	39±7	37±4	38±5
1500	Peak Amp.	28±13	25±10	26±12	22±11	20±8
	Latency	39±5	42±8	41±6	40±8	41±6
PND 60						
0	Peak Amp.	140±68	138±85	126±96	120±73	85±52
	Latency	43±4	42±3	42±5	42±5	41±6
10	Peak Amp.	126±65	149±88	138±90	107±70	80±54
	Latency	40±2	40±3	38±4	38±4	39±4
200	Peak Amp.	105±68	117±92	108±88	105±75	80±60
	Latency	43±7	40±9	39±7	38±5	37±7
1500	Peak Amp.	124±120	108±136	119±157	79±85	73±103
	Latency	41±4	39±4	37±4	39±6	38±5

a Data were extracted from Table 23 on pages 213-214 of the study report; n=14-16.

d. Learning and memory testing – No compound-related effects were noted at any dose in either sex in the passive avoidance or M-maze tests (Tables 16 and 17). In the passive avoidance test, acquisition was evident in both sexes as a marked increase in the latency to cross for the second trial compared to the first trial. Retention was evident as a protracted delay to cross within the 180-sec time limit of the first trail compared to the first trial on the first test day, and a reduced number of trials-to-criterion on the second test day compared to the first day. In the water maze test, acquisition was evident in both sexes as a progressive decrease in the average time to escape over successive trials. Retention was evident as a reduction in the number of trials-to-criterion and a shorter trial duration for the first trial compared to the first trial of acquisition.

TABLE 16. Mean (±SD) passive avoidance performance in F_1 rats [a]

Session/Parameter		Dose (ppm) 0	10	200	1500
Males					
Session 1 (PND 22) Learning	Trials to Criterion	2.9±0.3	3.3±0.8	3.1±0.4	3.1±0.6
	Latency Trial 1	42.7±44.2	46.0±40.3	49.9±44.0	56.5±42.3
	Latency Trial 2	180.0±0.0	166.5±39.5	165.7±40.0	180.0±8.0
	Failed to Meet Criterion	0	0	0	0
Session 2 (PND 29) Retention	Trials to Criterion	2.2±0.6	2.3±0.7	2.4±0.7	2.7±0.8
	Latency Trial 1	175.5±17.6	180.0±0.0	166.8±38.1	161.7±37.3
	Latency Trial 2	173.2±26.4	166.9±37.2	175.2±12.8	173.8±13.0
Females					
Session 1 (PND 22) Learning	Trials to Criterion	3.1±0.3	3.1±0.4	3.0±0.4	3.2±0.5
	Latency Trial 1	32.6±29.7	33.6±41.6	45.3±50.2	31.9±21.7
	Latency Trial 2	177.7±9.3	168.5±32.3	174.1±23.4	177.6±9.7
	Failed to Meet Criterion	0	0	0	0
Session 2 (PND 29) Retention	Trials to Criterion	2.3±0.6	2.1±0.4	2.5±0.7	2.8±0.9
	Latency Trial 1	168.9±31.5	168.4±33.0	155.6±52.0	164.7±34.4
	Latency Trial 2	173.8±24.9	180.0±0.0	166.6±35.5	157.7±48.9

a Data were extracted from Table 24 on pages 216-217 of the study report; n=15-16.

TABLE 17. Mean (±SD) water maze performance in F_1 rats [a]

Session/Parameter		Dose (ppm) 0	10	200	1500
Males					
Session 1 (PND 60±2) Learning	Trials to Criterion	8.2±3.1	7.1±2.1	9.5±4.2	8.2±3.2
	Trial 1 Errors	0.7±1.0	0.8±1.0	1.3±1.5	0.9±1.0
	Latency Trial 1	17.1±13.3	20.9±15.4	19.2±15.4	21.9±14.6
	Trial 2 Errors	0.4±0.7	0.8±0.9	0.4±0.6	0.4±0.7
	Latency Trial 2	18.8±14.2	21.4±11.2	11.5±6.7	15.9±13.9
	Failed to Meet Criterion	0	0	1	1
Session 2 (PND 67±2) Retention	Trials to Criterion	6.2±2.5	5.5±0.7	5.3±0.6	5.7±1.8
	Trial 1 Errors	0.4±0.6	0.8±1.7	0.1±0.4	0.1±0.4
	Latency Trial 1	9.5±7.8	11.1±11.4	6.6±4.8	8.5±5.6
	Trial 2 Errors	0.1±0.3	0.1±0.3	0.1±0.5	0.2±0.6
	Latency Trial 2	5.6±4.6	5.8±3.4	4.8±3.1	5.5±3.3
Females					
Session 1 (PND 60±2) Learning	Trials to Criterion	7.9±3.1	7.8±3.1	7.8±2.6	7.3±2.3
	Trial 1 Errors	0.4±0.6	0.9±0.8	0.6±0.7	0.9±1.0
	Latency Trial 1	12.0±4.6	19.3±13.5	12.3±5.5	19.9±17.5
	Trial 2 Errors	0.6±0.6	0.6±0.6	0.8±0.6	0.5±1.0
	Latency Trial 2	12.9±10.4	13.7±7.7	14.7±7.1	13.4±14.0
	Failed to Meet Criterion	0	1	1	0
Session 2 (PND 67±2) Retention	Trials to Criterion	5.8±1.4	7.0±3.1	6.5±2.0	5.4±0.9
	Trial 1 Errors	0.4±0.6	0.2±0.4	0.8±0.9	0.3±0.6
	Latency Trial 1	8.4±5.5	6.6±4.1	13.4±10.4	8.1±6.1
	Trial 2 Errors	0.1±0.3	0.1±0.5	0.5±1.0	0.2±0.8
	Latency Trial 2	4.9±3.3	5.2±3.5	7.6±7.6	5.4±6.9

a Data were extracted from Table 25 on pages 219-220 of the study report; n=15-16.

5. **Ophthalmology** – Although corneal opacities were noted on PND 50 (M: 2, 3, 1, 4; F: 0, 0, 1, 0; in the control, 10, 200, and 1500 ppm groups, respectively), it was stated that no compound-related ocular lesions were noted in either sex. These results differ from the results for clinical observations and the FOB, where compound-related corneal and general ocular opacities were evident in the 200 and 1500 ppm pups. It was stated that these differences were likely due to the limited sample used for ophthalmology and the relatively small number of animals affected.

6. **Postmortem results**

a. **Brain weights** – On PND 21, terminal body weights were decreased ($p \leq 0.05$) by 12-13% in the ≥200 ppm males and by 10% in the 1500 ppm females (Table 18). Absolute brain weights were also decreased ($p \leq 0.05$) in these groups by 6-9% and 6%, respectively. Relative (to body) brain weights were slightly increased in these groups (↑4-6%, not statistically significant) as well. It was stated that these findings on brain weight were considered to be secondary to the greater effect on body weight, representing partial but incomplete sparing of the brain tissue.

At termination (PND 75), terminal body weights were decreased ($p \leq 0.05$) by 12-13% in the ≥200 ppm males. Absolute brain weight was decreased ($p \leq 0.05$) by 6-7% in both sexes at 1500 ppm. Relative brain weight was increased ($p \leq 0.05$) by 13% in the 200 ppm males. It was stated that these findings were attributed to decreased body weight, especially during development and not a compound-related effect. Additionally, it was noted that the decreased brain weights evident in the 200 ppm males on PND 21 had recovered by study termination.

TABLE 18. Mean (±SD) brain weight data from perfused F_1 rats [a]

Parameter	Dose (ppm)			
	0	10	200	1500
Males				
PND 21				
Terminal body weight (g)	50.8±6.4	49.7±4.4	44.8±4.2*(↓12)	44.3±2.7*(↓13)
Brain weight (g)	1.436±0.08	1.400±0.04	1.344±0.05*(↓6)	1.308±0.08*(↓9)
Brain-to-body weight ratio	2.855±0.28	2.844±0.32	3.018±0.21	2.959±0.23
Termination				
Terminal body weight (g)	338.6±25.9	315.1±17.7	294.3±33.5*(↓13)	297.3±25.0*(↓12)
Brain weight (g)	1.859±0.07	1.868±0.09	1.809±0.06	1.722±0.09*(↓7)
Brain-to-body weight ratio	0.552±0.04	0.594±0.04	0.622±0.08*(↑13)	0.581±0.04
Females				
PND 21				
Terminal body weight (g)	46.6±3.1	49.0±3.0	44.7±1.9	42.1±3.4*(↓10)
Brain weight (g)	1.329±0.06	1.369±0.07	1.313±0.06	1.252±0.04*(↓6)
Brain-to-body weight ratio	2.859±0.16	2.804±0.21	2.944±0.15	2.989±0.24
Termination				
Terminal body weight (g)	196.9±15.4	199.2±18.2	191.5±15.8	190.5±21.0
Brain weight (g)	1.711±0.06	1.711±0.07	1.690±0.08	1.615±0.10*(↓6)
Brain-to-body weight ratio	0.873±0.07	0.866±0.09	0.886±0.07	0.854±0.08

a Data were extracted from Tables OW1K-SUM and OW2K-SUM on pages 844-848 of the study report; n=9-10. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).

* Statistically significantly different from controls at $p \leq 0.05$

b) Neuropathology

1. **Macroscopic examination** – No gross lesions were observed on PND 21 or at termination in either sex.

2. **Microscopic examination** – No compound-related microscopic lesions were observed in any tissue collected from the control or 1500 ppm animals on PND 21 or 70 (Tables 19a and 19b).

   On PND 21, decreases ($p \leq 0.05$) in morphometric measurements were noted in the following areas in the 1500 ppm females: (i) frontal cortex (↓13%); (ii) parietal cortex (↓8%); (iii) hippocampal gyrus (↓11%), and (iv) cerebellum height (↓7%). It was stated that the microscopic examination and gross brain measurements were reviewed with no

morphological changes found in any area of the brain. The increases ($p \leq 0.05$) noted in various morphometric measurements in the treated rats at 200 ppm were considered unrelated to treatment because they were all within the historical control range.

At termination (PND 70), the following decreases ($p \leq 0.05$) in morphometric measurements were noted at all doses: (i) frontal cortex (↓5-9% males and 6-9% females at 10 and 1500 ppm); (ii) parietal cortex (↓6-10% males and 3-10% females); and (iii) cerebellum height (↓4-7% males and 6-11% females). It was stated that the microscopic examination and gross brain measurements were reviewed with no morphological changes found in any area of the brain. With the exception of the frontal cortex in the females, all statistically significant measurements in the males and females were within the historical control ranges provided, and the measurements did not display a dose-response in any measured parameter. Therefore, these findings were not considered to be treatment-related.

TABLE 19a. Mean (±SD) morphometric brain measurements (mm) in male F_1 rats [a]

Parameter	Dose (ppm)				Historical Data [b]
	0	10	200	1500	
PND 21					
Cerebrum Length	13.75±0.36	13.55±0.32	13.47±0.32	13.38±0.41	NR
Cerebellum Length	7.17±0.36	7.15±0.23	7.02±0.26	6.89±0.18	NR
Frontal Cortex	1.61±0.01	NM	1.72±0.01*(↑7)	1.55±0.01	1.66-1.98
Parietal Cortex	1.72±0.00	NM	1.85±0.01*(↑8)	1.64±0.01	1.81-2.06
Caudate Putamen	2.85±0.01	NM	2.86±0.01	2.91±0.06	2.96-3.27
Hippocampal Gyrus	1.48±0.00	NM	1.54±0.01	1.49±0.01	1.44-1.71
Cerebellum Height	3.91±0.02	NM	3.90±0.02	3.77±0.04	4.03-4.56
Termination					
Cerebrum Length	14.77±0.31	14.59±0.47	14.58±0.36	14.44±0.23	NR
Cerebellum Length	7.74±0.37	7.90±0.38	7.57±0.44	7.53±0.21	NR
Frontal Cortex	1.80±0.00	1.64±0.01*(↓9)	1.71±0.01*(↓5)	1.64±0.02*(↓9)	1.58-1.91
Parietal Cortex	1.97±0.01	1.78±0.01*(↓10)	1.81±0.01*(↓8)	1.85±0.01*(↓6)	1.74-1.93
Caudate Putamen	3.41±0.00	3.41±0.02	3.41±0.02	3.36±0.02	3.12-3.51
Hippocampal Gyrus	1.78±0.03	1.84±0.01	1.74±0.01	1.77±0.01	1.57-1.74
Cerebellum Height	4.46±0.04	4.13±0.03*(↓7)	4.21±0.09*(↓6)	4.26±0.03*(↓4)	3.91-4.67

a Data were extracted from Tables OW1K-SUM and OW2K-SUM on pages 844 & 847, and BM1-SUM and BM2-SUM on pages 854, 855, 863, and 864 of the study report; n=9-10. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).

b Historical control data from approximately 14 studies were presented on pages 874-875 of the study report.

NM Not measured

NR Not reported

* Statistically significantly different from controls at $p \leq 0.05$

TABLE 19b. Mean (±SD) morphometric brain measurements (mm) in female F_1 rats [a]

Parameter	Dose (ppm)				Historical Data [b]
	0	10	200	1500	
PND 21					
Cerebrum Length	13.25±0.34	13.51±0.35	13.22±0.18	13.07±0.23	NR
Cerebellum Length	7.10±0.16	7.14±0.32	7.28±0.19	6.96±0.51	NR
Frontal Cortex	1.60±0.00	NM	1.80±0.02*(↑13)	1.40±0.01*(↓13)	1.64-2.00
Parietal Cortex	1.72±0.01	NM	1.81±0.01	1.58±0.01*(↓8)	1.82-2.03
Caudate Putamen	2.77±0.03	NM	2.99±0.01*(↑8)	2.70±0.03	2.88-3.30
Hippocampal Gyrus	1.49±0.01	NM	1.57±0.02	1.32±0.01*(↓11)	1.38-1.77
Cerebellum Height	3.94±0.06	NM	3.91±0.03	3.67±0.10*(↓7)	3.99-4.52
Termination					
Cerebrum Length	14.32±0.32	14.14±0.33	14.16±0.28	13.96±0.36	NR
Cerebellum Length	7.81±0.21	7.73±0.36	7.88±0.44	7.57±0.28	NR
Frontal Cortex	1.71±0.01	1.56±0.01*(↓9)	1.67±0.01	1.61±0.01*(↓6)	1.65-1.84
Parietal Cortex	1.87±0.00	1.68±0.00*(↓10)	1.74±0.00*(↓7)	1.81±0.00*(↓3)	1.75-1.88
Caudate Putamen	3.48±0.01	3.33±0.02*(↓4)	3.33±0.01*(↓4)	3.38±0.02	3.21-3.55
Hippocampal Gyrus	1.68±0.01	1.65±0.02	1.67±0.00	1.72±0.01	1.48-1.76
Cerebellum Height	4.47±0.04	4.06±0.10*(↓9)	3.96±0.04*(↓11)	4.19±0.04*(↓6)	4.01-4.52

a Data were extracted from Tables OW1K-SUM and OW2K-SUM on pages 845 & 848, and BM1-SUM and BM2-SUM on pages 858, 859, 868, and 869 of the study report; n=9-10. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).

b Historical control data from approximately 14 studies were presented on pages 874-875 of the study report.

NM Not measured

NR Not reported

* Statistically significantly different from controls at $p \leq 0.05$

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS= CONCLUSIONS – The investigators concluded that dietary administration of AE0172747 from GD 6 through LD 21 induced the following effects in the dams at ≥200 ppm: (i) decreased body weight during gestation and lactation; (ii) decreased weight gain during gestation; (iii) decreased food consumption during lactation; and (iv) corneal opacities during lactation. Additional findings at 1500 ppm were limited to clinical signs of toxicity (hunched posture, unthrifty appearance, discolored stool, and walking on tiptoes). In the offspring, the following decreases were noted at ≥200 ppm: (i) acoustic startle response; (ii) body weight during PND 11, 17, and 21 and during post-weaning; (iii) body weight gain during PND 0-21; and (iv) absolute brain weight in males on PND 21. Additionally at 1500 ppm, decreased absolute brain weight was noted in females on PND 21 and in both sexes at termination (PND 70). The decreased brain weight in the females on PND 21 was evident by general reduction in microscopic brain measurements.

B. REVIEWER COMMENTS – Reserved for EPA reviewer

C. STUDY DEFICIENCIES - None

APPENDIX I

BAYER CORP

MAIN FORM

Laboratory		Bayer Corp., Stilwell, KS		
Study No.	**MRID**	**TRX**	**Year**	**Citation**
1	45441302		2001	1. Sheets,L.P. and Lake,S.G. (2001) Method Validation Study for a Developmental Neurotoxicity Screen: Untreated (Nomative) and Perinatal Methimazole Treatment in Wistar Rats. Bayer Corporation, Stilwell, KS, Laboratory Study Number 98-982-RR, Feb 9, 2001. 973 p. MRID 45441302.
2	45441303		2001	2. Sheets,L.P. (2001) Historical Control and Method Validation Studies in rats for a Developmental Neurotoxicity Screening Battery (Auditory Startle Habituation and Cognitive Function (Passive Avoidance and Water Maze Conditioning). Bayer Corporation, Stilwell, KS, Laboratory Study Number 98-992-VV, 98-992-UM, 98-992-WC, 99-D82-AF, Feb 9, 2001. 191 p. MRID 45441303.
3	45464601		1999	3. Sheets,L.P. and Gilmore,R.G. (1999) Verification of Personnel Training to Perform a Functional Observational Battery with Rats. Unpublished study prepared by Bayer Corporation, Stilwell, KS. Laboratory Study Number 97-962-LG. September 16, 1999. 94 p. MRID 4544601
4	45464602		2000	4. Sheets,L.P. and Armintrout,G.L. (2000) A Motor Activity Historical Control and Method Validation Study using Triadimefon and Chlorpromazine in Wistar rats. Unpublished study prepared by Bayer Corporation, Stilwell, KS. Laboratory Study Number 97-482-OU. June 19, 2000. 56 p. MRID 45464602

Positive Control Review Form

Testing Laboratory	Bayer Co., Stilwell, KS	1. Sheets,L.P. and Lake,S.G. (2001) Method Validation Study for a Developmental Neurotoxicity Screen: Untreated (Nomative) and Perinatal Methimazole Treatment in Wistar Rats. Bayer Corporation, Stilwell, KS, Laboratory Study Number 98-982-RR, Feb 9, 2001. 973 p. MRID 45441302.	
Positive Control	Methimazole (d)		
Date of Positive Control Data	2001		
Species/Strain	Wistar rats		
QA Review (yes/no)	Yes	Date of Review	November 2002

Methods	Method Codes	Data Present?	Age Relevant?	Age (days)	Dose Levels	Sexes (m/f)	Group Size	Effects (y/n)	Comments
Dev Landmarks (PDX X)	PS,VO,EO,SR,PI, ASR	raw, means	yes	var	1	m,f	20	y	
FOB		yes	yes	4.1e+10	1	m,f	15	n	
Motor Activity	PH (Columbus)	yes	yes	13-17, 60	1	m,f	20	y	
Startle	CO	raw, means	yes	223860	1	m,f	20	y	
Learn/Memory	PA MZ	raw, means	yes	24-31 60-67	1	m,f	20	n y	
Std Histopath		yes	yes	11	1	m,f	10	n	
Morphometrics		raw, means	yes	11, 70	1	m,f	10	y, y	
Thyroid Hormone and Histopath		yes	yes	1170	1	m,f	10	y hormone y histopath	

Is data report adequate (individual data, methods, etc)?	Good example of data reporting. Separate submission in standard format. QA, summaries and raw data
Methods/Results	**Methods:** One dose of methimazole from GD16 to PND10 at 0.1 mg/ml in the drinking water. Standard methods used in the Bayer *lab for DNT studies.* **Results:** 14% decrease in maternal body weight and decrease in pup weights postnatally recovery in males not in females by PND~60. Most endpoints were affected. Exceptions included: no change in FOB, no change in PND24 passive avoidance, and no evidence of histopathology from standard subjective assessments. Motor activity was only affected on PND 13 and no other ages. No effect on startle on PND23, increases on PND 38 and 60 only in males. Only effect in males on learning phase in water maze test. No effects in females. There was an 84% decrease in T4 and a 16% decrease in T3.
Summary	Summary: It appears to be adequate data to support proficiency for developmental exposure to one agent. Problems include: only decrease seen in MA testing and only at one age, no increases; no effects on FOB measures, PND24 learning/memory testing or std histopath. Only effects in one sex in learning portion of water maze, no effects on retention testing. Note that other data from this lab support proficiency with adult motor activity. Overall Conclusion: Proficiency = marginal.

Positive Control Review Form

Testing Laboratory	Bayer Co., Stilwell, KS	2. Sheets,L.P. (2001) Historical Control and Method Validation Studies in rats for a Developmental Neurotoxicity Screening Battery (Auditory Startle Habituation and Cognitive Function (Passive Avoidance and Water Maze Conditioning). Bayer Corporation, Stilwell, KS, Laboratory Study Number 98-992-VV, 98-992-UM, 98-992-WC, 99-D82-AF, Feb 9, 2001. 191 p. MRID 45441303.	
Positive Control	1. 8-hydroxy-(di-n-propylamino) piperazine HBr (8OH-DPAT) 2. 1-m-chlorophenyl piperazine HCl (mCPP) 3. Scopolamine HBr		
Date of Positive Control Data	2001		
Species/Strain	Male and female Crl:Wistar(HAN,BR) rats		
QA Review (yes/no)	Yes	Date of Review	November 2002

Methods	Method Codes	Data Present?	Age Relevant?	Age (days)	Dose Levels	Sexes (m/f)	Group Size	Effects (y/n)	Comments
Dev Landmarks (PND X)									
FOB									
Startle	CO	yes	no	30	5 mg/kg	m	8	n	mCPP
Startle	CO	yes	no	32	0.5, 1.0 mg/kg	f	10	y	8OH-DPAT
Startle	CO	yes	no	30	0.25 mg/kg	m	8	n	8OH-DPAT
Learn/Memory	PA MZ	yes yes	n y	3556		m m,f	1212	yes no	scopolamine, MZ=water m-maze
Std Histo									
Morphometrics									

Is data report adequate (individual data, methods, etc)?	Data presentation is adequate.
Methods/Results	**Methods:** For startle used a fairly standard 50 trial habituation paridigm. Recorded peak amplitude. Testing was immediately post-dosing. Cognitive testing used an M-maze and one dose of scopolamine, 1.0 mg/kg administered between 30 and 60 minutes prior to testing. Training consisted of 15 trials. Retention testing was conducted 24 hours later. Variables were trials to criterion, number of errors and latency to goal. Passive avoidance used a standard paridigm: trials to criterion was maxed out at 15. **Results:** For startle there was no effect of the mCPP 8-OHDPAT caused an increase at the highest dose. Results for the water maze testing are not very good. There were no statistically significant effects on any measure in males, in females there was a decrease in latency (??), and an increase in the number that failed to meet criterion (controls = 2; scoploamine = 5). For passive avoidance the data are not very impressive. For the learning phase there was a small increase in the trials to criterion and a small decrease in the latency on trial 2, but not trial 1. For retention testing there was a small increase in trials to criterion, a decrease in latency on trial 1 and no effect on trial 2. Not very big effects compared to published literature on scopolamine and passive avoidance.
Summary	**Summary:** These data alone are marginal to non-acceptable as evidence of proficiency. There are only males for the PA testing and the effects of 1.0 mg/kg scopolamine are rather small. M-maze performance was not affected at all in males and affected only slightly in females. Startle shown to be increased, but not decreased by reference compounds used previously by the author. Overall Conclusion: Proficiency = not demonstrated for startle, PA, or M-maze.

Positive Control Review Form

Testing Laboratory	Bayer Co., Stilwell, KS	3. Sheets,L.P. and Gilmore,R.G. (1999) Verification of Personnel Training to Perform a Functional Observational Battery with Rats. Unpublished study prepared by Bayer Corporation, Stilwell, KS. Laboratory Study Number 97-962-LG. September 16, 1999. 94 p. MRID 45464601	
Positive Control	Carbaryl		
Date of Positive Control Data	1999		
Species/Strain	Wistar rats		
QA Review (yes/no)	Yes	Date of Review	November 2002

Methods	Method Codes	Data Present?	Age Relevant?	Age (days)	Dose Levels	Sexes (m/f)	Group Size	Effects (y/n)	Comments
Dev Landmarks (PND X)									
FOB		yes	yes/no	63	2 (15,30 mg/kg)	m	6	yes	carbaryl
Motor Activity									
Startle									
Learn/Memory									
Std Histopath									
Morphometrics									

Is data report adequate (individual data, methods, etc)?	Good example of data reporting. Separate submission in standard format. QA, summaries and raw data
Methods/Results	**Methods:** Standard FOB with ranking scales plus grip strength and footsplay. Carbaryl administered ip, 15-70 min prior to testing. Five technicians rated each animal and interobserver reliability was assessed. **Results:** Dose response was apparent. A variety of endpoints were affected as would be expected with carbaryl. Some endpoints were not affected. Good overall agreement between observers.
Summary	**Summary:** Data for FOB are inadequate: only data for males, small group size (n=6), one dose, only adults. Only one compound and not all endpoints affected. Same data as was submitted for adult neurotox studies. **Overall Conclusion:** unacceptable

Positive Control Review Form

Testing Laboratory	Bayer Co., Stilwell, KS	4. Sheets,L.P. and Armintrout,G.L. (2000) A Motor Activity Historical Control and Method Validation Study using Triadimefon and Chlorpromazine in Wistar rats.. Unpublished study prepared by Bayer Corporation, Stilwell, KS. Laboratory Study Number 97-482-OU. June 19, 2000. 56 p. MRID 45464602	
Positive Control	triadimefon, chlorpromazine		
Date of Positive Control Data	2000		
Species/Strain	Wistar rats		
QA Review (yes/no)	Yes	Date of Review	November 2002

Methods	Method Codes	Data Present?	Age Relevant?	Age (days)	Dose Levels	Sexes (m/f)	Group Size	Effects (y/n)	Comments
Dev Landmarks (PND X)									
FOB									
Motor Activity	F8	yes	yes/no	70	0, 200 mg/kg	m	12	yes	triadimefon
	F8	yes	yes/no	70	0, 2 mg/kg	m	12	yes	chlorpromazine
Learn/Memory									
Std Histopath									
Morphometrics									

Is data report adequate (individual data, methods, etc)?	Good example of data reporting. Separate submission in standard format. QA, summaries and raw data.
Methods/Results	**Methods:** Triadimefon (90 min prior to testing, po) and chlorpromazine (60 min prior, ip) were administered to 70 day old Wistar rats. Testing in figure-8 mazes lasted 90 min, summed in 10 min bins. Data analyzed by SAS. **Results:** Triadimefon resulted in increased activity of about 300% and a decrease in habituation. Chlorpromazine resulted in decreased activity, about 50% for total counts.
Summary	**Summary:** Triadimefon and chlorpromazine data are inadequate due to males only and only adults at 70 days of age. Data demonstrate ability to detect decreases and increases, as well as decreased habituation. Sensitivity is unknown due to lack of dose response. **Overall Conclusion:** Marginal.

APPENDIX II

Homogeneity and stability of AE 0172747 in Purina Mills Certified Rodent Diet 5002 Meal

In a revised homogeneity and stability study (MRID 46695739), AE 0172747 technical (94.0% a.i.; Batch Nos. OP2250027 and PFI 0215) was mixed with Purina Mills Certified Rodent Diet 5002 Meal at concentrations of 3 and 3000 ppm. The test material was dissolved in acetone prior to mixing with the diet. The acetone was allowed to evaporate from the dietary formulation after 10 minutes of mixing in a Hobart mixer. Samples for homogeneity (top, middle, bottom) and stability (7 days at room temperature and 7, 14, and 35 days frozen) were collected at each concentration. The samples were analyzed using liquid chromatographic/mass spectrometer/ mass spectrometer analysis (LC-MS/MS).

Homogeneity analysis (% relative standard deviation): 2.2-7.7%

Stability analysis (range as % of Day 0)
At room temperature for 7 days: 91-99%
Frozen for up to 35 days: 94-114%

The results indicate that AE 0172747 mixed with rodent ration at 3 and 3000 ppm was homogeneous, and stable at room temperature for 7 days or frozen for 35 days.

COMPLIANCE – Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.